Supplementary Materials

Supplementary Figures

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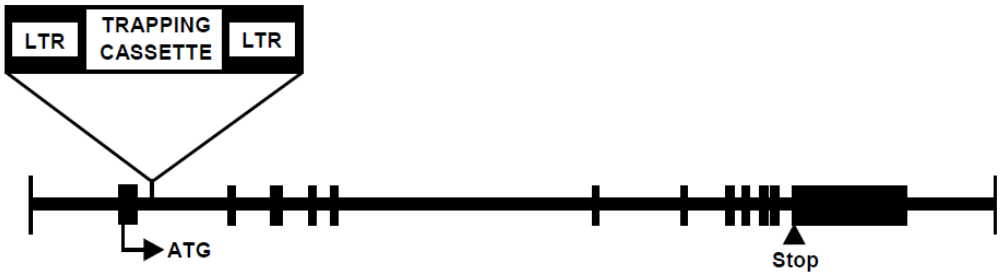

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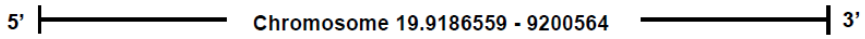

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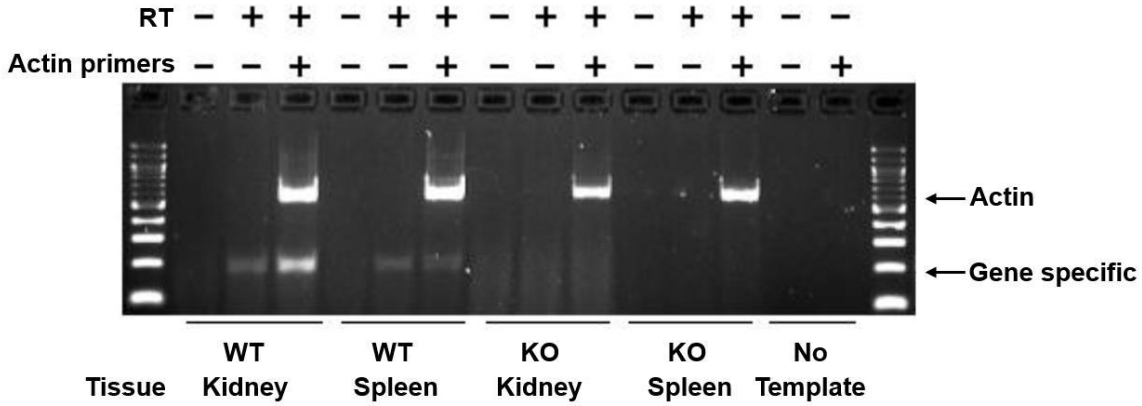
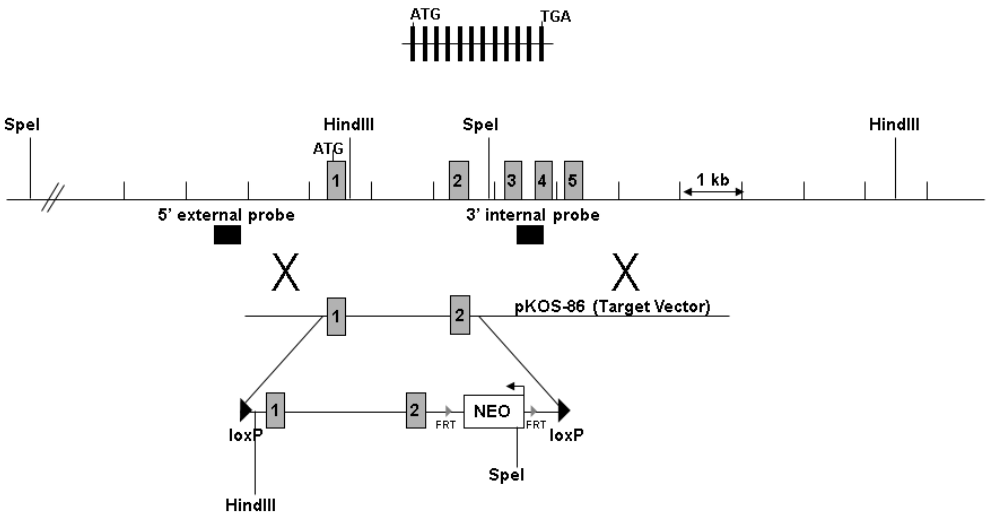
C



Supplementary Figure 1. Disruption of the Fads1 gene by gene trapping. **A**) Schematic diagram of the *Fads1* gene showing location of the retroviral gene-trap vector insertion in Omnibank embryonic stem cell clone OST118368 (accession number M146094). This clone was generated using a vector which disrupts endogenous gene transcription through action of a splice acceptor-neomycin phosphotransferase trapping cassette. Boxes denote coding exons and lines between boxes denote introns. Location of the vector insertion site in intron 1 is shown. LTR, viral long terminal repeat; ATG, translation start codon; Stop, translation stop codon. **B**) Mouse genomic sequence obtained by inverse PCR amplification of the pro-viral insertion site. An asterisk denotes the site of insertion. **C**) RT-PCR expression analysis of *Fads1* transcript. Endogenous *Fads1* transcript was detected in the kidney and spleen of WT but not KO mice. Primers complementary to sequence in *Fads1* exons 1 and 2 were used to amplify the gene-specific product. RT-PCR analysis using primers complimentary to the mouse *beta actin* gene (accession number M12481) was performed in the same reaction to serve as an internal amplification control. Lanes labeled as No Template represent control reactions. RT, reverse transcriptase.

A



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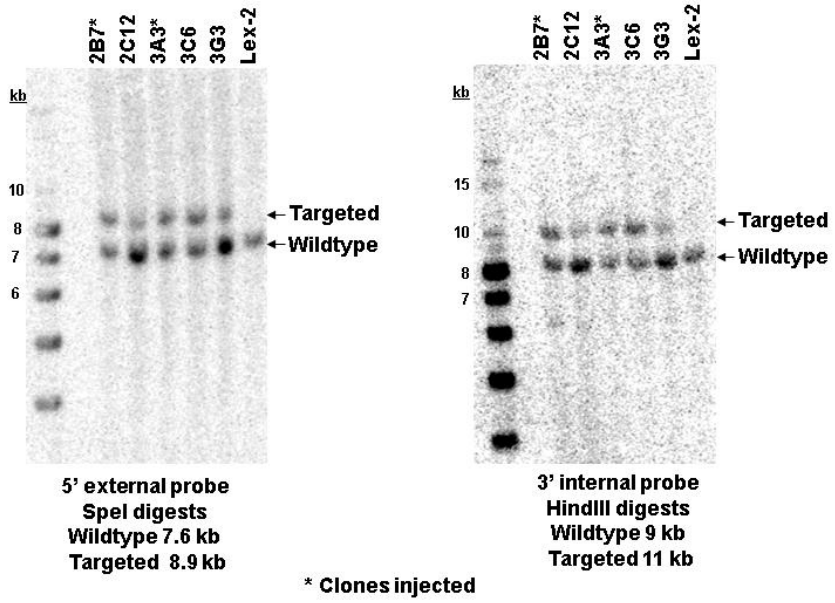
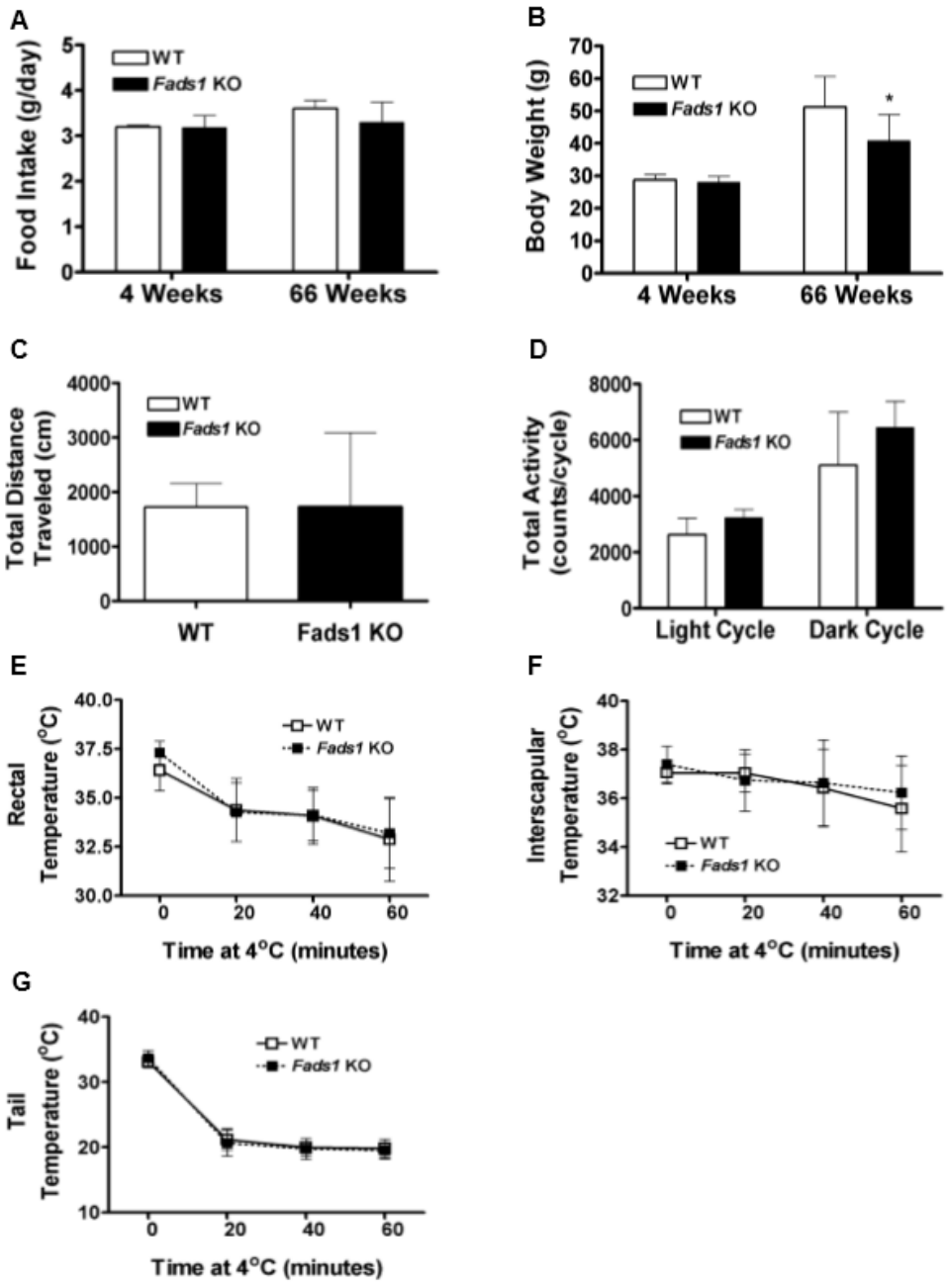
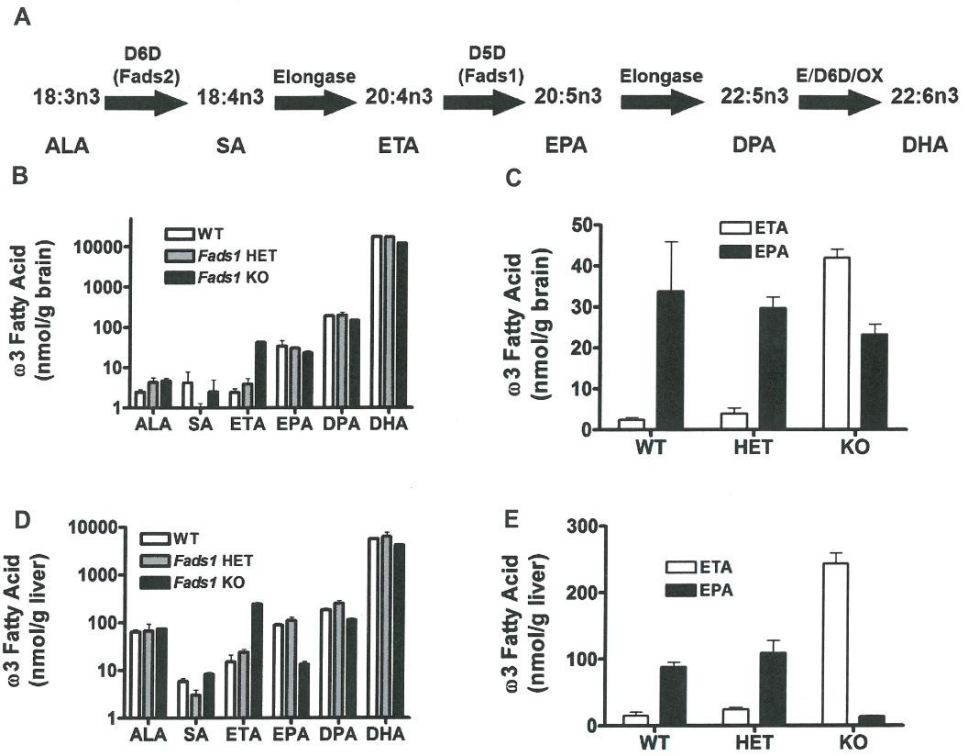


Supplementary Figure 2. Targeted disruption of the *Fads1* gene locus by homologous recombination. A) Targeting strategy used to disrupt the *Fads1* locus. Homologous recombination (represented by **X**) between the targeting vector and the *Fads1* gene results in the substitution of a LoxP flanked exons 1 and 2 along with a Frt flanked selection cassette. **B)** Southern hybridization indicating proper gene targeting in the embryonic stem cell clones. Clones 2B7 and 3A3 were used for blastocyst injections; Lex-2 represents untransfected embryonic stem cell DNA.



Supplementary Figure 3. Effect of *Fads1* KO on food intake, body weight, activity levels and cold tolerance. A) Intake of 45% HFD by group-housed *Fads1* KO mice and their WT littermates (2-3 mice/cage, 4 cages/genotype) between 4 and 6 weeks of age and again between 66 and 67 weeks of age. **B)** Body weights of these mice at 4 and 66 weeks of age. KO mice different from WT mice, * $p < 0.05$. **C)** Total distance traveled by chow-fed, 11 week-old *Fads1* KO mice (N = 8) and their WT littermates (N = 4) measured in an open field chamber. **D)** Total activity levels of 45% HFD-fed, 63 week-old male *Fads1* KO mice (n=8) and their WT littermates (n=8), measured over 24 hours in the ER-4000 physiological measurement system (see Materials and methods). In a cold tolerance assay, 45% HFD-fed, 23-55 week-old male *Fads1* KO mice (n=18) and their WT littermates (n=17) had their **E)** rectal, **F)** interscapular and **G)** tail temperatures measured at baseline (Time=0) and then after being individually housed for 20, 40 and 60 minutes at 4°C.



Supplementary Figure 4. Levels of ω3 fatty acids present in phospholipids isolated from brain and liver of *Fads1* KO, HET and WT mice. Individual ω3 fatty acids were quantitated in 5 phospholipid fractions (cardiolipin, phosphatidylserine, phosphatidylethanolamine, phosphatidylcholine and lysophosphatidylcholine) isolated from brain and liver samples of 44 week-old *Fads1* KO (n=2), HET (n=2) and WT (n=2) littermate mice (see Materials and methods for details); data from the 5 phospholipid fractions were then combined to yield a single pooled value for each individual ω3 fatty acid in brain or liver tissue of each mouse. **A)** Enzymes and fatty acids that comprise the ω3 fatty acid pathway. D5D, delta 5 desaturase; D6D, delta 6 desaturase; Fads1, fatty acid desaturase 1; Fads2, fatty acid desaturase 2; E, elongase; OX, peroxisomal oxidation; ALA, α-linolenic acid; SA, stearidonic acid; ETA, eicosatetraenoic acid; EPA, eicosapentaenoic acid; DPA, n3 docosapentaenoic acid; DHA, **B)** Brain levels of ω3 fatty acids along the ω3 fatty acid pathway. **C)** Brain levels of ETA and EPA, the substrate and product, respectively, of *Fads1*. **D)** Liver levels of ω3 fatty acids along the ω3 fatty acid pathway. **E)** Liver levels of ETA and EPA. All data are presented as nmol of ω3 fatty acid present in the 5 phospholipid fractions isolated from 1 gram of tissue.

Supplementary Tables

Supplementary Table 1. Body composition of *Fads1* KO and WT mice at 15-20 weeks of age

Cohort	GT	N	Diet	Body weight (g)	Body fat (g)	% Body fat	LBM (g)
1M	WT	11	60% HFD	40.1 ± 4.2	13.0 ± 4.0	31.7 ± 7.4	27.1 ± 1.2
1M	KO	12	60% HFD	35.4 ± 4.0*	8.8 ± 2.6**	24.6 ± 5.8*	26.5 ± 2.5
1F	WT	11	60% HFD	30.0 ± 3.2	9.7 ± 3.1	31.8 ± 7.7	20.3 ± 1.6
1F	KO	8	60% HFD	28.9 ± 2.7	8.2 ± 1.8	28.2 ± 4.0	20.7 ± 1.0
2M	WT	7	45% HFD	41.6 ± 6.6	11.5 ± 4.8	26.6 ± 8.5	30.1 ± 2.7
2M	KO	8	45% HFD	36.6 ± 3.7	8.6 ± 2.0	23.2 ± 3.4	28.0 ± 1.9
3M	WT	11	Chow	35.6 ± 5.3	5.5 ± 3.1	14.8 ± 6.0	30.1 ± 2.7
3M	KO	11	Chow	30.1 ± 3.6**	3.4 ± 1.6*	10.9 ± 4.1	26.7 ± 2.5**
4M	WT	17	45% HFD	40.0 ± 4.9	10.8 ± 2.9	26.6 ± 5.1	29.2 ± 2.8
4M	KO	15	45% HFD	33.8 ± 3.0***	6.9 ± 1.9***	20.4 ± 3.9***	26.8 ± 2.0**
4F	WT	7	45% HFD	32.9 ± 5.8	11.8 ± 4.1	35.0 ± 6.8	21.1 ± 2.1
4F	KO	7	45% HFD	27.2 ± 43.2*	6.5 ± 2.2*	23.5 ± 5.6**	20.7 ± 1.5
5M	WT	8	45% HFD	40.2 ± 5.4	11.2 ± 4.0	27.1 ± 7.1	29.0 ± 2.1
5M	KO	15	45% HFD	37.1 ± 4.6	6.7 ± 3.1**	17.6 ± 6.0**	30.3 ± 2.0
5F	WT	13	45% HFD	28.2 ± 2.8	7.0 ± 3.0	24.4 ± 8.3	21.2 ± 1.5
5F	KO	9	45% HFD	25.2 ± 4.5	4.5 ± 2.8	17.0 ± 6.6*	20.7 ± 2.3
6M	WT	15	45% HFD	50.4 ± 6.1	18.0 ± 4.0	35.3 ± 4.7	28.2 ± 2.0
6M	KO	17	45% HFD	37.9 ± 6.5***	10.1 ± 4.5***	25.6 ± 6.9***	23.7 ± 2.2***
6F	WT	14	45% HFD	38.1 ± 7.6	15.8 ± 5.8	40.1 ± 7.8	19.4 ± 2.0
6F	KO	15	45% HFD	32.9 ± 6.8	11.8 ± 4.8	34.5 ± 7.3	18.3 ± 2.1

GT = genotype; N = number of mice; M = Male, F = female; LBM, lean body mass; HFD, high fat diet.
KO different from WT, *$P < 0.05$; ** $P < 0.01$; ***$P < 0.001$.

Supplementary Table 2. Body composition of 45% HFD-fed *Fads1* KO, HET and WT mice at 16-22 weeks of age

Cohort	GT	N	Body weight (g)	Body fat (g)	% Body fat	LBM (g)
7M	WT	11	41.7 ± 4.2	12.0 ± 2.1	28.8 ± 3.5	29.7 ± 3.2
7M	HET	9	43.1 ± 5.3	14.6 ± 2.5	33.8 ± 2.7	28.5 ± 3.3
7M	KO	14	35.5 ± 5.0* ++	8.2 ± 3.4** +++	22.4 ± 6.7* +++	27.3 ± 2.4
7F	WT	6	32.2 ± 7.4	11.3 ± 6.3	32.4 ± 13.4	20.9 ± 1.3
7F	HET	11	31.2 ± 5.9	9.9 ± 4.0	30.7 ± 7.4	21.3 ± 2.4
7F	KO	10	28.1 ± 4.5	7.3 ± 3.1	25.3 ± 7.2	20.8 ± 2.1
8M	WT	10	42.2 ± 5.8	12.0 ± 2.6	28.1 ± 3.0	30.3 ± 3.6
8M	HET	8	38.8 ± 6.9	9.7 ± 4.1	24.1 ± 7.4	29.1 ± 3.9
8M	KO	12	34.2 ± 3.8**	6.2 ± 2.6***	17.8 ± 5.9***	28.0 ± 2.7
8F	WT	5	26.8 ± 3.8	6.5 ± 2.7	23.5 ± 6.8	20.3 ± 1.1
8F	HET	7	30.6 ± 5.0	9.0 ± 3.8	28.3 ± 7.0	21.7 ± 1.3
8F	KO	5	25.8 ± 4.0	4.9 ± 3.0	17.8 ± 8.9	21.0 ± 1.6
9M	WT	4	43.5 ± 3.0	15.9 ± 2.2	36.5 ± 2.9	27.6 ± 1.3
9M	HET	4	45.4 ± 7.4	15.1 ± 5.1	32.5 ± 6.0	30.3 ± 2.3
9M	KO	4	37.2 ± 3.0	9.8 ± 3.0	26.2 ± 7.1	27.3 ± 2.0
9F	WT	4	32.8 ± 3.8	11.8 ± 3.5	35.3 ± 6.4	21.1 ± 0.6
9F	HET	3	29.1 ± 3.2	8.5 ± 3.0	28.7 ± 7.8	20.7 ± 2.0
9F	KO	4	28.9 ± 1.8	7.7 ± 2.8	26.2 ± 8.4	21.2 ± 1.4

GT = genotype; N = number of mice; M = Male, F = female; LBM, lean body mass.
KO different from WT, *$P < 0.05$; ** $P < 0.01$; ***$P < 0.001$.
KO different from HET, ++$P < 0.01$; +++$P < 0.001$.

Supplementary Table 3. Body composition of 16 week old *Fads1* mice generated by homologous recombination

Cohort	GT	N	Diet	Body weight (g)	Body fat (g)	% Body fat	LBM (g)
1hrM	WT	10	45% HFD	40.2 ± 3.8	13.5 ± 1.3	33.7 ± 1.1	26.6 ± 2.6
1hrM	KO	11	45% HFD	31.6 ± 3.1***	5.7 ± 2.2***	17.8 ± 5.5***	25.9 ± 1.9
1hrF	WT	8	45% HFD	27.5 ± 6.1	7.5 ± 4.9	25.4 ± 11.1	20.0 ± 2.1
1hrF	KO	11	45% HFD	24.0 ± 4.3	4.5 ± 2.0	17.9 ± 5.2	19.5 ± 2.5

GT = genotype; N = number of mice; M = Male; F = Female; hr, homologous recombination; LBM, lean body mass.
KO different from WT, *$P < 0.05$; ** $P < 0.01$; ***$P < 0.001$.

Supplementary Table 4. Body composition of 45% HFD-fed *Fads1* KO and WT mice at age > 1 year

Cohort	GT	Age (wks)	N	Body weight (g)	Body fat (g)	% Body fat	LBM (g)
5M	WT	66	6	56.1 ± 7.3	21.8 ± 5.2	38.4 ± 4.7	34.3 ± 2.7
5M	KO	66	7	47.4 ± 12.2	14.4 ± 8.4	28.5 ± 10.0*	33.0 ± 4.2
1hrM	WT	74-75	9	67.4 ± 6.2	30.3 ± 5.3	44.6 ± 3.9	37.1 ± 1.3
1hrM	KO	74-75	9	39.7 ± 7.0***	9.8 ± 5.6***	23.4 ± 8.9***	29.8 ± 3.3***
1hrF	WT	74-75	7	44.5 ± 18.7	18.9 ± 14.5	37.1 ± 15.6	25.6 ± 4.7
1hrF	KO	74-75	4	35.4 ± 6.8	12.2 ± 3.3	34.1 ± 5.1	23.2 ± 4.0

GT = genotype; N = number of mice; wks = weeks; M = Male; F = Female; hr = homologous recombination; LBM, lean body mass.
KO different from WT, *$P < 0.05$; ** $P < 0.01$; ***$P < 0.001$.

Supplementary Table 5. Serum total triglyceride, total cholesterol, ALT and AST levels and liver weights in Fads*1* KO mice

Cohort	GT	Age	N	TG (mg/dL)	Chol (mg/dL)	Liver Wt (g)	ALT (U/L)	AST (U/L)
5M	WT	30 wks	8	170 ± 41	248 ± 74			
5M	KO	30 wks	15	121 ± 46*	154 ± 40***			
6M	WT	30 wks	10	90 ± 27	251 ± 48	2.8 ± 0.7	240 ± 119	232 ± 118
6M	KO	30 wks	10	67 ± 20*	143 ± 42***	1.4 ± 0.4***	74 ± 56***	125 ± 53*
6F	WT	30 wks	10	47 ± 12	188 ± 41	1.4 ± 0.3	138 ± 103	268 ± 346
6F	KO	30 wks	10	55 ± 32	141 ± 41*	1.3 ± 0.2	58 ± 33*	265 ± 275
7M	WT	37 wks	9	158 ± 37	219 ± 48			
7M	KO	37 wks	10	139 ± 32	174 ± 15*			
7F	WT	37 wks	6	127 ± 24	158 ± 33			
7F	KO	37 wks	9	116 ± 43	121 ± 25*			
8M	WT	46 wks	6	121 ± 49	215 ± 73			
8M	KO	46 wks	10	91 ± 37	135 ± 41*			
8F	WT	46 wks	3	85 ± 34	106 ± 10			
8F	KO	46 wks	3	77 ± 18	92 ± 25			
1hrM	WT	31 wks	10	193 ± 42	242 ± 46			
1hrM	KO	31 wks	11	141 ± 49*	166 ± 43***			
1hrF	WT	31 wks	8	111 ± 20	106 ± 27			
1hrF	KO	31 wks	11	98 ± 16	115 ± 48			

GT = genotype; N = number of mice; TG, total triglyceride; Chol, total cholesterol; Wt, weight; ALT, alanine aminotransferase; AST, aspartate aminotransferase; wks, weeks
KO different from WT, *$P < 0.05$; ***$P < 0.001$.

Supplementary Table 6. Body weight and lipid levels in *Fads1* KO and WT mice on an *ApoE* KO background

		Males		Females	
Measurement	Time on western diet	WT (17)	*Fads1* KO (16)	WT (21)	*Fads1* KO (15)
Body weight (g)	4 weeks	29.0 ± 3.6	27.8 ± 4.4	22.9 ± 2.7	21.8 ± 2.0
Body weight (g)	8 weeks	36.1 ± 4.6	30.8 ± 4.0**	25.3 ± 2.4	23.7 ± 3.2
Body weight (g)	12 weeks	41.8 ± 6.2	33.8 ± 4.9***	28.0 ± 3.3	25.3 ± 3.7*
Total Chol (ng/mL)	4 weeks	983 ± 244	1037 ± 212	870 ± 138	950 ± 132
Total Chol (ng/mL)	8 weeks	1149 ± 218	1210 ± 271	1066 ± 248	1113 ± 245
Total Chol (ng/mL)	12 weeks	1129 ± 247	1061 ± 274	1015 ± 217	1002 ± 243
Total TG (ng/mL)	4 weeks	213 ± 51	193 ± 59	179 ± 60	173 ± 52
Total TG (ng/mL)	8 weeks	177 ± 64	181 ± 66	157 ± 68	141 ± 48
Total TG (ng/mL)	12 weeks	197 ± 53	182 ± 54	155 ± 53	135 ± 45

(N) = number of mice; Chol = cholesterol.
KO different from WT, *P < 0.05; ** P < 0.01; ***P < 0.001.